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Patentanmeldung Nr. Patent application No. Demande de brevet n°

03075255.4

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Production of 5-ribonucleotides

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The application was transferred from the above mentioned application to:
DSM IP Assets B.V. Heerlen, the Netherlands

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Production of 5'-ribonucleotides

Field of the invention

The present invention relates to a composition comprising 5'-ribonucleotides and a process for the production of this composition.

Autolytic yeast extracts are concentrates of the soluble materials obtained from yeast after disruption of the cells and digestion (lysis) of the polymeric yeast material. The active yeast enzymes released in the medium after cell disruption are responsible for the lysis. This type of yeast extracts, which are rich in amino acids, are used in the food industry as basic taste providers. The amino acids present in the yeast extract add a bouillon-type brothy taste to the food.

Hydrolytic yeast extracts, on the other hand, are concentrates of the soluble materials obtained from yeast after disruption of the cells and digestion (lysis), when to the yeast suspension during lysis additional proteases, and/or peptidases and especially nucleases are added. During this process 5'-ribonucleotides of guanine (5'-GMP), uracil (5'-UMP), cytosine (5'-CMP) and adenine (5'-AMP) are formed. When adenylic deaminase is added to the mixture, 5'-AMP is transformed into 5'-inosine mono phosphate (5'-IMP). The hydrolytic yeast extracts obtained by this method are therefore rich in 5'-ribonucleotides, especially rich in 5'-GMP and 5'-IMP. Often yeast extracts are also rich in mono sodium glutamate (MSG). 5'-IMP, 5'-GMP and MSG are known for their flavour enhancing properties. They are capable of enhancing the savoury and delicious taste in certain types of food. This phenomenon is described as 'mouthfeel' or umami.

These yeast extracts rich in 5'-ribonucleotides and, optionally, rich in MSG, are usually applied in soups, sauces, marinades, and flavour seasonings.

JP 51106791 describes a process for the purification of RNA using ultrafiltration of a yeast extract followed by precipitation of RNA by salting out at pH2. The precipitated RNA is isolated by centrifugation. The isolated RNA is dispersed in water and the pH is brought to 6. Macromolecular inpurities are insolubilised. The suspended matter is subsequently coagulated by heating, and flocks are removed by filtration or

centrifugation whereafter the clear fluid is dried. Although UF filtratic
Japanese patent document is used many additional steps are neede
commercial attractive RNA product which makes this process exper

The present invention has overcome the disadvantages of s
discloses a process which is very simple and cost-effective and ther
very attractive. Surprisingly the present invention combines the sep
yeast extracts with the conversion of separated RNA into 5'-ribonuc
process. In this way it is possible to produce 5'-ribonucleotides havir
relatively simple and thus commercially very attractive process.

The present invention provides a composition comprising at
NaCl free dry matter base) of 5'-ribonucleotides, preferably at least
at least 75% of 5'-ribonucleotides. Preferably this composition also
Moreover the present invention provides for a process for the produ
composition of the invention. The present invention provides a proc
composition enriched in 5'-ribonucleotides which comprises

- treating the cells walls of microbial cells to form treated microbial c
content is liberated,
- separating the RNA liberated from the microbial cells from the othe
cell materials, and,
- converting the separated RNA into 5'-ribonucleotides.

With the term "5'-ribonucleotide" it is herewith intended either the fre
a salt thereof.

The amounts of 5'-ribonucleotide, 5'-GMP, 5'-AMP and 5'-IMP in the
invention (%w/w) is based on NaCl free dry matter of the compositio
and 5'-IMP are expressed as 2Na.7Aq on NaCl free dry solids. NaC
that the composition should not contain NaCl, but means that for the
NaCl is excluded in the calculation. Glutamate is given as glutamic
on glutamic acid on NaCl free dry matter of the compostion.

The compositions of the invention preferably comprises glut
ratio of glutamate to 5'-ribonucleotides is less than 0.1 preferably le
preferably less than 0.01 and/or whereby this ratio is more than 0.0
composition comprises 0.01 to 10%, preferably 0.05 to 5%, more pr
w/w (on dry matter base) of glutamate based on NaCl free dry matte

The compositions of the invention comprises preferably more 5'-GMP than the sum of 5'-IMP and 5'-AMP(on dry matter weight); and is preferably obtained from yeast. Commercially available yeast extracts all contain less 5'-GMP than the sum of 5'-IMP and 5'-AMP whereas the 5'-ribonucleotide composition of present invention obtained from a yeast extract contains more 5'-GMP than the sum of 5'-IMP and 5'-AMP. This relatively higher amount of 5'-GMP in such a composition is advantageous because this results in a stronger flavor enhancement power of this composition compared with presently commercially available yeast extracts (T.Nagodawithana, Savours Flavours, (1995) edited by Esteekay associates, Inc, Wiscosin, USA, page 302)

The production process of the present invention may start with a fermentation broth of the microorganism or microbial cells in question. Fermentation processes are known in the art. In some cases the fermentation broth can be concentrated for example by centrifugation before use in the present process. For example cream yeast (Baker's yeast) can be used which is concentrated to 15-27wt%. The microbial cells are preferably treated to inactivate the microbial enzymes present in the cells. In autolytic microbial extracts in general the RNA is broken down and separation of this RNA is therefore less attractive.

All kind of microorganism can be used as natural source of RNA. Bacterial and fungal micro organisms are known which are suitable for food and feed applications. The preferred microorganisms are those that have the status of being food-grade, thus microorganisms which can be safely applied for human food consumption.

Examples hereof are fungi such as *Trichoderma* or *Aspergillus* and yeast a.o. from the genus *Saccharomyces*, *Kluyveromyces* and Candida. Especially yeast RNA is useful in many food applications.

Examples of suitable bacterial microorganism are lactic acid bacteria.

The fermentation broth is generally obtained from strains with a high RNA content. In this way a high amount of 5'-ribonucleotides is generated during the hydrolytic process. Yeast strains belonging to the genera *Saccharomyces*, *Kluyveromyces* and *Candida* are advantageously used. Yeast strains belonging to the genus *Saccharomyces*, for example to the strain *Saccharomyces cerevisiae* are preferred. Although yeast with high RNA content are preferred, also yeast with low RNA content can be used. These yeasts can advantageously be converted into compositions having a high 5'-ribonucleotides content or into yeast extracts having high 5'-ribonucleotides content, higher than could be

expected on basis of the RNA content of the starting yeast or higher than yeast extracts produced in the presently available extraction processes.

Inactivation of microbial enzymes in the fermentation broth is possible with for example a heat shock, for example 5 to 10 minutes at a temperature of 80° - 97°C. This treatment should inactivate at least the enzymes that break down the RNA of the microorganism. So after this treatment the RNA of the microorganism will not be broken until in a later stage, for example when an enzyme is added for that purpose.

In order to release the cell contents from the cells, the cells can be treated chemically, mechanically or enzymatically. Preferably enzymes are used for this solubilisation or cell wall lysing step. For example a protease such as endoprotease can be used. The reaction conditions for the protease depend on the enzyme used. In general the microbial cells are enzymatically treated for 1-24 hours, at a pH between 4 and 10 and a temperature of 40 –70°C. After the chemical or enzymatic treatment, the chemicals or enzymes should preferably be neutralised. For example the enzymes should be inactivated. Inactivation of the enzymes can be done by pH treatment or preferably by a heat treatment whereby the enzyme is inactivated and the treated microbial cells at least for the RNA content are not substantially altered. After liberation of the cell contents a composition is obtained which comprises microbial cell walls, carbohydrates, proteins, RNA, minerals, lipids, and vitamins. The present invention makes use of the fact that RNA will remain intact during the inactivation process of the enzymes of the microbial cells as well as during the cell wall treatment. Subsequently the RNA liberated from the microbial cells is separated from the other soluble microbial cell materials and then the RNA is converted into 5'-ribonucleotides.

RNA can preferably be separated from the other microbial cell materials by making a first separation of the solids present (such as cell walls) and other soluble materials including RNA, proteins, carbohydrates, minerals, lipids and vitamins. For this first separation, for example centrifugation or filtration can be applied. Subsequently the soluble RNA can be separated from other soluble materials, for example by ultra filtration (UF). On laboratory scale the separation of RNA can be performed in several ways with the goal to obtain very high pure RNA whereby small amounts have to be isolated. These methods are often very labour-intensive and expensive. The present process provides a process especially useful for large scale separation of RNA or 5'-ribonucleotides which process allows the use of RNA or 5'-ribonucleotides in food and

feed applications on a commercially attractive and large scale. The present invention provides a process which results in a good purity of 5'nucleotides and rather low losses. By large scale is meant that fermentation in fermentors of more than 10 m^3 are performed to produce the microbial cells which is the starting material for the process of the invention. In general fermentors will have a size of 50 to 250 m^3. The broth of this fermentation is then used for the process of the invention. So in general fermentation broths of more than 10 m^3 can be converted in the compositions of the present invention.

Hereafter the separated RNA can be converted into 5'-ribonucleotides.

In case UF is used to separate RNA from the other soluble cell material, in general filters of 10 to 50 kD, preferably 20 to 50 kD, can be used. In general the larger size filters allow a higher flow through the filter, but might result in small losses and/or less pure product.

RNA can be converted into 5'-ribonucleotides enzymatically. 5'phosphodiesterase (5'-Fdase) can be used. 5' phosphodiesterase can be obtained from a microbial or a vegetable source (for example a malt root extract). An example of commercial available microbial product is Enzyme RP-1 produced by Amano. Deaminase, for example adenyl deaminase, can be used to convert AMP into IMP. An example of a commercial available product is Deaminase 500 produced by Amano.

The 5'-ribonucleotides fraction, which is a very high nucleotide product, is clean in taste which has several applications in food/feed. This composition of the invention is free of the specific taste or smell of the microorganism, for example does not taste yeasty in case of *Saccharomyces* starting materials or does not taste sweet in case of Candida starting materials. This composition when applied in food product in proper amounts will not give bouillon/brothy notes.

According to an embodiment of the invention the 5'-ribonucleotide fraction can be added to conventional yeast extracts in every desired ratio. For example yeast extract having a 5'-ribonucleotide content can be obtained which was not available sofar. All fractions, compositions and mixtures thereof according to the present invention have in common that natural sources are used which make them very suitable for food addition.

Example 1

40,000 kg cream yeast (dry solids is 18.2 %) was heat treated in a continuous flowthrough heat exchanger for 10 minutes at 95 °C in order to inactivate all yeast

enzyme activity. Subsequently this inactivated yeast is treated batchwise for 6 hours with Pescalase (endo-protease from *Bacillus licheniformis,* DSM N.V., The Netherlands) at pH 8.0 and 62 °C. Thereafter, the protease is inactivated by heat treatment for 1 hour at 70 °C (batchwise) and the pH is lowered to 5.3 with hydrochloric acid. The solids are removed from the reaction mixture by continuous centrifugation. The remaining supernatant is ultra filtered on a 50 kD ultra filter to separate the high molecular weight fraction (including RNA) form low molecular weight material like inorganic components, vitamins, carbohydrates (like trehalose), free amino acids, peptides and small proteins. The high molecular weight fraction (Retentate UF1) is then incubated batchwise for 15 hours at pH 5.3 and 65 °C with the enzyme 5'-phosphodiesterase in order to hydrolyse the RNA into 5'-ribonucleotides. Next, the liberated 5-'AMP is converted into 5'-IMP by the enzyme deaminase during a 2.5 hour incubation at pH 5.1 and 55 °C. Finally, the reaction mixture is ultra filtered again on the 50 kD filter. The filtrate dry solids consist mainly of 5'-ribonucleotides (filtrate UF2).

Data of the extraction process is presented is Table 1.

Table 1: nucleotide extraction process data

	Amount (kg)	Dry solids (%w/w)	Dry solids (kg)	RNA ** (%)	5'GMP *** (%)	5'IMP *** (%)	Glutamic acid ** (%)
Cream yeast	40000	18,20	7280	8,2			
Supernatant *	54100	8,77	4750	10,3			5,5
Retentate UF1	8120	7,80	632	72,5			0,4
Filtrate UF2 *	10160	3,98	404	0,0	24,5	24,1	0,5

* including wash liquid
** % on NaCl free dry solids
*** espressed as 2Na.7aq on NaCl free dry solids

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CLAIMS

1. A composition comprising at least 55%w/w (on NaCl free, dry matter base) of 5'-ribonucleotides, preferably at least 65%, more preferably at least 75% of 5'-ribonucleotides which further comprises glutamate whereby preferably the ratio of glutamate to 5'-ribonucleotides is less than 0.1, more preferably less than 0.05, and most preferably less than 0.01 and/or whereby this ratio is preferably more than 0.001.
2. A composition according to claim 1 which comprises 0.01 to 10%, preferably 0.05 to 5%, more preferably 0.1 to 2% w/w (on dry matter base) of glutamate.
3. A composition according to claim 1 which comprises more 5'-GMP than the sum of 5'-IMP and 5'-AMP (on dry matter weight).
4. A process to produce a composition enriched in 5'-ribonucleotides which comprises
   - treating the cells walls of microbial cells to form treated microbial cells whereby the cell content is liberated,
   - separating the RNA liberated from the microbial cells from the other soluble microbial cell materials, and,
   - converting the separated RNA into 5'-ribonucleotides.
5. A process of claim 4 whereby before treating the microbial cells, the microbial enzymes of microbial cells are inactivated.
6. A process according to claim 4 or 5 whereby the cell walls are treated enzymatically, chemically or mechanically preferably by a protease.
7. A process according to any one of claims 4 to 6 whereby before the separation of the RNA liberated from the cells from the soluble microbial cell materials, the solids from the microbial cells are removed.
8. A process according to claim 7, wherein the removal of the solids is done by centrifugation or filtration.
9. A process according to any one of claim 4 to 8 where the separation of the RNA liberated from the microbial cells is done by ultra filtration.

10. A process according to any one of claim 4 to 9 wherein the separated RNA is enzymatically converted into 5'-ribonucleotides preferably by 5'-Fdase or by 5'-Fdase and deaminase

11. A process according to any one of claim 4 to 10 whereby the 5'-ribonucleotides are further purified by removal of compounds having a MW of more than the 5'-ribonucleotides .

12. A process according to claim 11 whereby the removal of compounds having a MW of more than the 5'-ribonucleotides is done by ultra filtration.

13. Use of the composition of claims 1 to 3 or of a composition enriched in 5'-ribonucleotides produced by a process of claim 4 to 12, in food and feed products or food and feed intermediate products.

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Production of 5'-ribonucleotides

ABSTRACT

The present invention describes a composition comprising at least 55%w/w (on salt free dry matter base) of 5'-ribonucleotides, preferably at least 65%, more preferably at least 75% of 5'-ribonucleotides.